PRELIMINARY EXPERIMENTS ON THE EFFECT OF COLD ON VARIOUS DISEASES IN SMALL ANIMALS

BY

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Prefatory Note by R. Ross

Some years ago Sir Edwin Durning-Lawrence, Bart., offered funds to the Liverpool School of Tropical Medicine for the purpose of experimenting on the effect of cold on Yellow Fever—a subject in which he had long been interested owing to various reports which had come to his knowledge. The School found it difficult to give effect to his wishes, and the matter was allowed to drop.

For a long time I have felt that much experimental work still remains to be done regarding the cure of parasitic diseases, especially by such simple natural agencies as heat and cold. Most of the animal parasites occur most frequently in persons who live in warm climates, that is to say, they are accustomed to live in hosts who themselves live under conditions favourable to the said parasites. There may be reasons for thinking that if these conditions are abruptly altered the result will be harmful to the parasites, just as abrupt changes are apt to be harmful to higher organisms. Of course, the temperature of the patient's body is not markedly changed by alteration of external temperature; but, nevertheless, certain changes may be produced in his blood or tissues which are likely to be inimical to parasites living in him. I cannot point to any very definite prima facie evidence in favour of this view, but some general observations may be mentioned. For example, it is a common custom to send patients suffering from malaria to temperate climates, as to the hills in India. From my own clinical experience I certainly think it is easier to treat malaria in

England than in the tropics, and easier to treat it in the cool season in the tropics than in the very hot season—that is to say, I think that a given dose of quinine will produce least effect in the hot climates in which the parasites are most easily propagated. Moreover, if we make a careful study of statistics in India, we often observe a sudden rise in the malaria rate at the end of the cold season, long before any new brood of Anophelines has hatched out. True, this rise may be partly explained by the emergence of old Anophelines from hibernation; but it may also be due to relapses caused by the greater heat. There are also many observations on record, especially in Italy, suggesting that season affects the plasmodia, especially the sexual forms, quite apart from the proliferation of the carrying agents. Human trypanosomiasis occurs in persons in very hot climates, and may just possibly be retarded if such persons are removed to cold ones. In one case of complete cure known to me, marked improvement occurred when the patient was subjected to considerable cold, accompanied by healthy exercise and open air in Scotland. Dysentery seems to improve much more rapidly in cool climates than in hot ones. Possibly the effect may only be a slight one; but it is well worth while to study whether there is any effect at all, and if so how much—because anything that concerns so important a matter as the treatment of disease is worth considering. Still greater improvement might be effected in many maladies, especially diseases of the respiratory system, and of the skin, if cold is combined with a very dry and pure atmosphere, and, of course, with other forms of treatment.

Last year, therefore, I asked Sir Edwin Durning-Lawrence whether he would not assist us to carry out researches on these points in Liverpool. He assented at once, and suggested that I should ask his friend, Sir Alfred Haslam, the head of the great refrigerating works at Derby to construct a chamber capable of being kept at the requisite temperature. The result was that the Haslam Engineering Company made such a chamber at the University Laboratories of this School It was completed on September 10th, 1909, and after a few interruptions, has been kept continuously running ever since. Major C. L Williams was put in charge of the experiments from early in September until the 13th April, 1910, since when the work has been conducted by Dr. J. G. Thomson. At the time when Dr. J. G.

Thomson commenced his labours, Dr. David Thomson and myself had just elaborated what we call Enumerative Methods* for the study of parasitic diseases, and these have now been employed for the Cryotherapy work. In this paper, however, we record only the preliminary experiments conducted by the old methods—which were the only ones used by Major Williams.

Our warm thanks are due to Sir Edwin Durning-Lawrence for his munificence regarding this extensive line of research, and to Sir Alfred Haslam for the great interest which he has taken in the matter.

Summary of Experiments from the 10th September, 1909, to 13th April, 1910

By Major C. L. WILLIAMS, I.M.S.

The chamber is 12 feet long by 7 feet wide by $6\frac{3}{4}$ feet high, with a cubic content of about 540 feet, and can be kept at any temperature between about 15° F. (−9·4° C.) and 150° F. (65·5° C.). The motor was worked usually from about 7.45 a.m. to 5.30 p.m., but had to be stopped at intervals, perhaps for an hour or two at a time whenever 20° F. was reached. Usually it rose during the night to 36° or 38° F., or thereabouts, and that would be the initial temperature at 7.30 a.m. at the time of starting the machinery. The temperature was reduced by an ammonia compressor worked by a 6-h.p. motor and a fan driving in air through a chamber in which a saturated solution of calcium chloride was kept constantly trickling over corrugated iron plates.

In theory the air is not supposed to change or be refreshed by additions of outside air, but apart from the rush of air into the chamber each time the door was opened, a procedure necessary several times a day, the absence of smell and the practically inappreciable amount of CO_2 and organic matter in the air when tested chemically point very strongly to a rapid removal of respiration products: and, moreover, the animals in the chamber at no time showed any signs of intoxication by impurities of re-breathed air.

The low humidity of the air in the chamber is no doubt a strong factor in the results. It varied usually from about 50 to 60 per cent.,

* Vide page 261 of this number.

TABLE SHEWING THE COURSE OF VARIOUS TRYPANOSOMIASES UNDER ORDINARY AND UNDER COLD CHAMBER CONDITIONS UP TO FEBRUARY 21, 1910

Trypanosome	Animal infected	Number of animals		Incubation period in days			Period lived in days			Failed to shew infection	Remarks
				Longest	Shortest	Average	Longest	Shortest	Average		
T. lewisi	Rat	13	Cold chamber 6	25	4	14	127 (Still alive)	20	40	2	One still alive (21.2.10) shewed trypanosomes for 35 days. Its weight has risen from 85 to 163 grams in 127 days (its coat, however, is much longer, and it was probably young when received). Two died after 45 and 53 days, of epidemic pneumonia.
			Controls 7	12	4	8	112 (Still alive)	21	46	3	Two died after 12 and 69 days respectively of epidemic pneumonia. One is still alive.
T. equiperdum	Rat	28	Cold chamber 14	20	6	13·4	36	12	25	1	Note that the sole failure was in the cold chamber (? aborted infection). One died soon after inoculation.
			Controls 14	24	8	17·8	38	21	26·8	—	
T. brucei	Guinea-pig	15	Cold chamber 8	36	4	18	62	20	37·1	—	One died soon after inoculation.
			Controls 7	35	5	17·1	57	20	34·1	—	
T. evansi	Guinea-pig	6	Cold chamber 3	21	18	19·5	26	24	25	1	
			Controls 3	10	Not known	0	21	Not known	21	1	One died in five days (? cause), before any trypanosomes had shewn in the peripheral blood.

NOTE.—Some of these animals were removed for ten days to the animal house whilst the cold chamber was under repair, and for that amount of time were subject only to ordinary temperatures.

NOTE.—One 'lewisi' rat is still alive (21.2.10) in the cold chamber, and seems lively and well. It has been in 277 days, and still shews undoubted trypanosomes.

being higher on days when the humidity outside was high, and being raised each time the door had to be opened to admit of feeding, taking observations, etc. To the observer it proved very invigorating, comparable probably to the air in the interior of Canada. The animals in the chamber, too, seemed more active and more interested in their surroundings than the controls which were kept in a greenhouse, artificially heated, but with a very varying temperature and with the humidity of the outside air. Curiously enough, the animals in the control house threw out actually longer coats than those in the cold chamber, and their appetites were markedly less.

The animals subjected to the influence of the cold were guinea-pigs, rats and mice. The animals were placed in cages, well bedded and well fed, fat and proteid especially being provided in their diet.

The diseases brought under the influence of the cold were chiefly various trypanosomiases, with, in addition, tubercle (bovine), cancer (in mice), tetanus, and spirochaetosis, and the results during over eight months' observation are tabulated below. During the eight months there were occasional intermissions for a few days for repairs to machinery, etc., but they amounted to but quite a few days in all.

In the table it will be noted that four animals died of pneumonia, an epidemic of which, unfortunately, occurred at one period and carried off several of the animals under observation. It was probably introduced with some wild rats placed at first near the controls, but affected both controls and cold chamber subjects equally.

The influence of *T. lewisi* is, in any case, difficult to ascertain as rats recover spontaneously from this affection; but it will be noted that the only animal which failed to show trypanosomes after inoculation with *T. brucei* was one in the cold chamber. The dose in all cases was, of course, the same, in terms of c.c. for the animal observed and its control, but the actual number of trypanosomes injected must vary within very wide limits, and this factor will influence the incubation periods. Whereas it seems to be somewhat delayed in *T. lewisi* it varies but little in the other trypanosomes. There was a small prolongation of life in the Ngana and Caderas cases, but this did not hold good for *T. lewisi* and Dourine.

Twelve mice were inoculated with cancer, and six put into the cold chamber; five lived 50, 51, 56, 70 and 90 days respectively, whereas of their six controls, two lived 56 and 76 days respectively.

Of the remainder, three controls failed to develop tumours, and one each in the control and cold chambers died within a few days of inoculation.

Six mice were also put into the cold chamber uninoculated as controls, and of them two are still alive (21 Feb.), well and vigorous, after 120 days, showing that cold *per se* had no bad effect on them; whilst two were eaten by their comrades after respectively 24 and 42 days' residence in the cold chamber, and two died of natural causes in 56 and 62 days.

Some mice were also infected with *Spirochaeta duttoni*, but no appreciable influence on the course of this disease could be observed in them.

Five guinea-pigs with tetanus were placed under observation, a case of a boy cured of that disease whilst being treated in a refrigerator having been reported by Crane ('St. Louis Medical Review,' 7th July, 1906). Of the tetanus guinea-pigs, one injected with $\frac{1}{500}$ c.c. of a culture and placed in the cold chamber, died in $9\frac{1}{2}$ days, but it is very doubtful if it died of tetanus; two injected with $\frac{1}{1500}$ and $\frac{1}{1000}$ c.c. respectively and kept as controls never showed any symptoms; whilst two injected with $\frac{1}{500}$ c.c. and placed one in the cold chamber and one as control died with similar symptoms in a similar time—something over 50 hours.

An Englishman suffering from sleeping sickness of some three months' duration, and contracted in N. Rhodesia, was submitted to a course of treatment in the chamber from January 5 onwards to the end of that month. Ordinarily he came from the hospital in which he was by cab about 10 a.m., and rested in the chamber, well wrapped up, till about 3.30 p.m. In all he had a course (interrupted for some days) of twelve days, or some 53 hours in all, of cold air. The temperature was gradually decreased till 24° F. was reached. He himself said he felt much better for the treatment, which acted on him as a tonic; but in the short period he was under observation no visible diminution was apparent in the number of trypanosomes in the blood. He was getting atoxyl simultaneously at his hospital. Several times he walked back—a distance of fully $1\frac{1}{2}$ miles—after his cold chamber rest; but since February 1 he has not been able to leave the Hospital, and has therefore been unable to utilise the action of cold in altering his metabolism, and thereby the culture media within his body of *T. gambiense*.

As regards increase or loss of weight in the animals under observation, most of them were young, and their weights, of course, increased by mere growth. Heavier coats also added to the weight. Of those on which accurate data can be founded the results are as follow:— Number of animals: Rats, 19; guinea-pigs, 8. Rats: Gained weight, 14; lost weight, 5. Guinea-pigs: Gained weight, 6; lost weight, 2.

Animal	Cold Chamber		Controls		Total
	Gained weight	Lost weight	Gained weight	Lost weight	
T. lewisi (rats)	3	1	1	1	6
T. equiperdum (rats)	6	1	4	2	13
T. brucei (guinea-pigs)	3	—	2	—	5
Tubercle	—	1	1	—	2
T. evansi	—	—	—	1	1
Total	12	3	8	4	27

Put shortly, all the animals in the cold chamber gained weight except three, of which one was a case of tubercle: showing, on the whole, and as far as the small figures go, a favourable influence on their general nutrition. But these figures are amply supported by the obvious increase in weight of practically all the animals in the chamber, though their immaturity in most cases makes the actual results in grams of weight gained of less value from the point of view of influence of cold *per se*.

Finally, as regards the temperatures of the animals, no constant effect could be observed in the influence of the cold chamber, and those in and those out varied equally irregularly, with no permanent ratio between the one and the other.

Note on 12 April, 1910. The result of further experiments carried out since February 21 and bringing the observation period up to over eight months is as follows:—

Six guinea-pigs were inoculated with bovine tubercle (0·1 mgr. culture) on the 27th November, and three placed in the cold chamber

and three outside, with, of course, uninoculated controls in both cases. The uninoculated controls throve and call for no remark, except that one in the cold chamber died after 34 days of no obvious cause, there being only a little lung congestion evident in post mortem. Of the inoculated, all are still alive, and those in the cold chamber have now been in it 137 days, and seem quite fit and well. One female has aborted twice, but whether from the cold, the tubercle, or other cause, cannot be definitely stated. Only three were apparently mature at the time of inoculation, and their weights in the case of two inoculated have risen in one case from 400 to 653 grams, and fallen in the other from 772 to 747; whilst the third, uninoculated, has risen from 622 to 794. Their temperatures have not shown any very striking variations; but the swelling over the site of inoculation burst fully a month sooner in the two controls outside, in which rupture has occurred, than in the one in the cold chamber, which developed suppuration; and this, as far as it goes, points to some diminution of the virulence of the bacilli in the cold chamber.

Four guinea-pigs have since (22 March) been inoculated with human tubercle, and three of them are at present under observation, one having died soon after inoculation.

Six guinea-pigs were inoculated with *T. gambiense* on the 14th December, and, as usual, placed three in the cold chamber and three outside as controls. All failed to show trypanosomes in the peripheral blood. One in the cold chamber died 16 days after inoculation, but no trypanosomes were found in the heart's blood post mortem, and it probably died of other causes. The other five were again inoculated on the 4th March, and again failed to show trypanosomes. But a third inoculation on March 30 has been successful and interesting, inasmuch as, whereas the three outside showed parasites in their peripheral bloods on the fifth day, of the two inside one only showed them very scantily on the eighth day, and has not shown them since, the other has so far not shown them at all: a result pointing strongly towards at least a delayed development in those in the colder air.

Several rats have been inoculated with cultures of pneumococcus, but so far no marked difference has been observable in their symptoms, whether in or out of the chamber, and none have died so that they remain under observation, and the final result will only become apparent later.